SECTION 14
TEST METHOD

MYSID, *MYSIDOPSIS BAHIA*, SURVIVAL,
GROWTH, AND FECUNDITY TEST
METHOD 1007.0

14.1 SCOPE AND APPLICATION

14.1.1 This method, adapted in part from USEPA (1987d), estimates the chronic toxicity of effluents and receiving waters to the mysid, *Mysidopsis bahia*, during a seven-day, static renewal exposure. The effects include the synergistic, antagonistic, and additive effects of all the chemical, physical, and additive components which adversely affect the physiological and biochemical functions of the test organisms.

14.1.2 Daily observations on mortality make it possible to also calculate acute toxicity for desired exposure periods (i.e., 24-h, 48-h, 96-h LC50s).

14.1.3 Detection limits of the toxicity of an effluent or pure substance are organism dependent.

14.1.4 Brief excursions in toxicity may not be detected using 24-h composite samples. Also, because of the long sample collection period involved in composite sampling and because the test chambers are not sealed, highly volatile and highly degradable toxicants present in the source may not be detected in the test.

14.1.5 This test is commonly used in one of two forms: (1) a definitive test, consisting of a minimum of five effluent concentrations and a control, and (2) a receiving water test(s), consisting of one or more receiving water concentrations and a control.

14.2 SUMMARY OF METHOD

14.2.1 *Mysidopsis bahia* 7-day old juveniles are exposed to different concentrations of effluent, or to receiving water in a static system, during the period of egg development. The test endpoints are survival, growth (measured as dry weight), and fecundity (measured as the percentage of females with eggs in the oviduct and/or brood pouch).

14.3 INTERFERENCES

14.3.1 Toxic substances may be introduced by contaminants in dilution water, glassware, sample hardware, and testing equipment (see Section 5, Facilities, Equipment, and Supplies).

14.3.2 Improper effluent sampling and handling may adversely affect test results (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

14.3.3 The test results can be confounded by (1) the presence of pathogenic and/or predatory organisms in the dilution water, effluent, and receiving water, (2) the condition of the brood stock from which the test animals were taken, (3) the amount and type of natural food in the effluent, receiving water, or dilution water, (4) nutritional value of the brine shrimp, *Artemia* nauplii, fed during the test, and (5) the quantity of brine shrimp, *Artemia* nauplii, or other food added during the test, which may sequester metals and other toxic substances, and lower the DO.

14.3.4 pH drift during the test may contribute to artifactual toxicity when ammonia or other pH-dependent toxicants (such as metals) are present. As pH increases, the toxicity of ammonia also increases (see Subsection 8.8.6), so upward pH drift may increase sample toxicity. For metals, toxicity may increase or decrease with increasing pH. Lead and copper were found to be more acutely toxic at pH 6.5 than at pH 8.0 or 8.5, while nickel and zinc were more toxic at pH 8.5 than at pH 6.5 (USEPA, 1992). In situations where sample toxicity is confirmed to be artifactual and due to pH drift (as determined by parallel testing as described in Subsection 14.3.4.1), the regulatory authority may allow for control of sample pH during testing using procedures outlined in Subsection 14.3.4.2. It should be noted that artifactual toxicity due to pH drift is not likely to occur unless pH drift is large (more than 1 pH unit) and/or the concentration of some pH-dependent toxicant in the sample is near the threshold for toxicity.

14.3.4.1 To confirm that toxicity is artifactual and due to pH drift, parallel tests must be conducted, one with controlled pH and one with uncontrolled pH. In the uncontrolled-pH treatment, the pH is allowed to drift during the test. In the controlled-pH treatment, the pH is maintained using the procedures described in Subsection 14.3.4.2. The pH to be maintained in the controlled-pH treatment (or target pH) will depend on the objective of the test. If the objective of the WET test is to determine the toxicity of the effluent in the receiving water, the pH should be maintained at the pH of the receiving water (measured at the edge of the regulatory mixing zone). If the objective of the WET test is to determine the absolute toxicity of the effluent, the pH should be maintained at the pH of the sample after adjusting the sample salinity for use in marine testing.

14.3.4.1.1 During parallel testing, the pH must be measured in each treatment at the beginning (i.e., initial pH) and end (i.e., final pH) of each 24-h exposure period. For each treatment, the mean initial pH (e.g., averaging the initial pH measured each day for a given treatment) and the mean final pH (e.g., averaging the final pH measured each day for a given treatment) must be reported. pH measurements taken during the test must confirm that pH was effectively maintained at the target pH in the controlled-pH treatment. For each treatment, the mean initial pH and the mean final pH should be within ± 0.3 pH units of the target pH. Test procedures for conducting toxicity identification evaluations (TIEs) also recommend maintaining pH within ± 0.3 pH units in pH-controlled tests (USEPA, 1996).

14.3.4.1.2 Total ammonia also should be measured in each treatment at the outset of parallel testing. Total ammonia concentrations greater than 5 mg/L in the 100% effluent are an indicator that toxicity observed in the test may be due to ammonia (USEPA, 1992).

14.3.4.1.3 Results from both of the parallel tests (pH-controlled and uncontrolled treatments) must be reported to the regulatory authority. If the uncontrolled test meets test acceptability criteria and shows no toxicity at the permitted instream waste concentration, then the results from this test should be used for determining compliance. If the uncontrolled test shows toxicity at the permitted instream waste concentration, then the results from the pH-controlled test should be used for determining compliance, provided that this test meets test acceptability criteria and pH was properly controlled (see Subsection 14.3.4.1.1).

14.3.4.1.4 To confirm that toxicity observed in the uncontrolled test was artifactual and due to pH drift, the results of the controlled and uncontrolled-pH tests are compared. If toxicity is removed or reduced in the pH-controlled treatment, artifactual toxicity due to pH drift is confirmed for the sample. To demonstrate that a sample result of artifactual toxicity is representative of a given effluent, the regulatory authority may require additional information or additional parallel testing before pH control (as described in Subsection 14.3.4.2) is applied routinely to subsequent testing of the effluent.

14.3.4.2 The pH can be controlled with the addition of acids and bases and/or the use of a CO_2-controlled atmosphere over the test chambers. pH is adjusted with acids and bases by dropwise adding 1N NaOH or 1N HCl (see Subsection 8.8.9). The addition of acids and bases should be minimized to reduce the amount of additional ions (Na or Cl) added to the sample. pH is then controlled using the CO_2-controlled atmosphere technique. This may be accomplished by placing test solutions and test organisms in closed headspace test chambers, and then injecting a predetermined volume of CO_2 into the headspace of each test chamber (USEPA, 1991b; USEPA, 1992); or by placing test chambers in an atmosphere flushed with a predetermined mixture of CO_2 and air (USEPA, 1996). Prior experimentation will be needed to determine the appropriate CO_2/air ratio or the appropriate volume of CO_2 to inject. This volume will depend upon the sample pH, sample volume, container volume, and sample constituents. If more than 5% CO_2 is needed, adjust the solutions with acids (1N HCl) and then flush the headspace with no more than 5% CO_2 (USEPA, 1992). If the objective of the WET test is to determine the toxicity of the effluent in the receiving water, atmospheric CO_2 in the test chambers is adjusted to maintain the test pH at the pH of the receiving water (measured at the edge of the regulatory mixing zone). If the objective of the WET test is to determine the absolute toxicity of the effluent, atmospheric CO_2 in the test chambers is adjusted to maintain the test pH at the pH of the sample after adjusting the sample salinity for use in marine testing. USEPA (1996) and Mount and Mount (1992) provide techniques and guidance for controlling test pH using a CO_2-controlled atmosphere. In pH-controlled testing, control treatments must be subjected to all manipulations that sample treatments are subjected to. These manipulations must be shown to cause no lethal or sublethal effects on control organisms. In pH-controlled testing, the pH also must be measured in each treatment at the beginning and end of each 24-h exposure period to confirm that pH was effectively controlled at the target pH level.

14.4 SAFETY

14.4.1 See Section 3, Health and Safety.

14.5 APPARATUS AND EQUIPMENT

14.5.1 Facilities for holding and acclimating test organisms.

14.5.2 Brine shrimp, *Artemia*, culture unit -- see Subsection 14.6.12 below and Section 4, Quality Assurance.

14.5.3 Mysid, *Mysidopsis bahia*, culture unit -- see Subsection 14.13 below. This test requires a minimum of 240 7-day old (juvenile) mysids. It is preferable to obtain the test organisms from an in-house culture unit. If it is not feasible to culture mysids in-house, juveniles can be obtained from other sources, if shipped in well oxygenated saline water in insulated containers.

14.5.4 Samplers -- automatic sampler, preferably with sample cooling capability, that can collect a 24-h composite sample of 5 L.

14.5.5 Environmental chamber or equivalent facility with temperature control (26 ± 1°C).

14.5.6 Water purification system -- Millipore Milli-Q®, deionized water or equivalent.

14.5.7 Balance -- Analytical, capable of accurately weighing to 0.00001 g.

14.5.8 Reference weights, Class S -- for checking performance of balance. Weights should bracket the expected weights of the weighing pans and weighing pans plus organisms.

14.5.9 Drying oven -- 50-105°C range, for drying organisms.

14.5.10 Desiccator -- for holding dried organisms.

14.5.11 Air pump -- for oil-free air supply.

14.5.12 Air lines, and air stones -- for aerating cultures, brood chambers, and holding tanks, and supplying air to test solutions with low DO.

14.5.13 Meters, pH and DO -- for routine physical and chemical measurements.

14.5.14 Tray -- for test vessels; approximately 90 X 48 cm to hold 56 vessels.

14.5.15 Standard or micro-Winkler apparatus -- for determining DO and checking DO meters.

14.5.16 Dissecting microscope (350-400X magnification) -- for examining organisms in the test vessels to determine their sex and to check for the presence of eggs in the oviducts of the females.

14.5.17 Light box -- for illuminating organisms during examination.

14.5.18 Refractometer or other method -- for determining salinity.

14.5.19 Thermometers, glass or electronic, laboratory grade -- for measuring water temperatures.

14.5.20 Thermometers, bulb-thermograph or electronic-chart type -- for continuously recording temperature.

14.5.21 Thermometer, National Bureau of Standards Certified (see USEPA Method 170.1, USEPA, 1979b) -- to calibrate laboratory thermometers.

14.5.22 Test chambers -- 200 mL borosilicate glass beakers or non-toxic 8 oz disposable plastic cups or other similar containers. Forty-eight (48) test vessels are required for each test (eight replicates at each of five effluent concentrations and a control). To avoid potential contamination from the air and excessive evaporation of test solutions during the test, the chambers should be covered with safety glass plates or sheet plastic (6 mm thick).

14.5.23 Beakers or flasks -- six, borosilicate glass or non-toxic plasticware, 2000 mL for making test solutions.

14.5.24 Wash bottles -- for deionized water, for washing organisms from containers and for rinsing small glassware and instrument electrodes and probes.

14.5.25 Volumetric flasks and graduated cylinders -- Class A, borosilicate glass or non-toxic plastic labware, 50-2000 mL for making test solutions.

14.5.26 Separatory funnels, 2-L -- Two-four for culturing *Artemia*.

14.5.27 Pipets, volumetric -- Class A, 1-100 mL.

14.5.28 Pipets, automatic -- adjustable, 1-100 mL.

14.5.29 Pipets, serological -- 1-10 mL, graduated.

14.5.30 Pipet bulbs and fillers -- PROPIPET®, or equivalent.

14.5.31 Droppers, and glass tubing with fire polished edges, 4 mm ID -- for transferring organisms.

14.5.32 Forceps -- for transferring organisms to weighing pans.

14.5.33 NITEX® or stainless steel mesh sieves ($\leq$150 µm, 500-1000 µm, 3-5 mm) -- for concentrating organisms.

14.5.34 Depression glass slides or depression spot plates -- two, for observing organisms.

14.6 REAGENTS AND CONSUMABLE MATERIALS

14.6.1 Sample containers -- for sample shipment and storage (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

14.6.2 Data sheets (one set per test) -- for data recording (Figures 2, 7, and 8).

14.6.3 Tape, colored -- for labeling test chambers.

14.6.4 Markers, waterproof -- for marking containers, etc.

14.6.5 Weighing pans, aluminum -- to determine the dry weight of organisms.

14.6.6 Buffers, pH 4, pH 7, and pH 10 (or as per instructions of instrument manufacturer) -- for standards and calibration check (see USEPA Method 150.1, USEPA, 1979b).

14.6.7 Membranes and filling solutions -- for dissolved oxygen probe (see USEPA Method 360.1, USEPA, 1979b), or reagents for modified Winkler analysis.

14.6.8 Laboratory quality assurance samples and standards -- for the above methods.

14.6.9 Reference toxicant solutions -- see Section 4, Quality Assurance.

14.6.10 Reagent water -- defined as distilled or deionized water that does not contain substances which are toxic to the test organisms (see Section 5, Facilities, Equipment, and Supplies).

14.6.11 Effluent, receiving water, and dilution water -- see Section 7, Dilution Water, and Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests. Dilution water containing organisms that might prey upon or otherwise interfere with the test organisms should be filtered through a fine mesh net (with 150 µm or smaller openings).

14.6.11.1 Saline test and dilution water -- The salinity of the test water must be in the range of 20‰ to 30‰. The salinity should vary by no more than ± 2‰ among the chambers on a given day. If effluent and receiving water tests are conducted concurrently, the salinities of these tests should be similar.

14.6.11.2 The overwhelming majority of industrial and sewage treatment effluents entering marine and estuarine systems contain little or no measurable salts. Exposure of mysids to these effluents will require adjustments in the salinity of the test solutions. It is important to maintain a constant salinity across all treatments. In addition, it may be desirable to match the test salinity with that of the receiving water. Two methods are available to adjust salinities – a hypersaline brine (HSB) derived from natural seawater or artificial sea salts.

14.6.11.3 HSB has several advantages that make it desirable for use in toxicity testing. It can be made from any high quality, filtered seawater by evaporation, and can be added to the effluent or to deionized water to increase the salinity. Brine derived from natural seawater contains the necessary trace metals, biogenic colloids, and some of the microbial components necessary for adequate growth, survival, and/or reproduction of marine and estuarine organisms, and may be stored for prolonged periods without any apparent degradation. However, if 100‰ HSB is used as a diluent, the maximum concentration of effluent that can be tested is 80% effluent at 30‰ salinity and 70% effluent at 30‰ salinity.

14.6.11.3.1 The ideal container for making brine from natural seawater is one that (1) has a high surface to volume ratio, (2) is made of a non-corrosive material, and (3) is easily cleaned (fiberglass containers are ideal). Special care should be used to prevent any toxic materials from coming in contact with the seawater being used to generate the brine. If a heater is immersed directly into the seawater, ensure that the heater materials do not corrode or leach any substances that would contaminate the brine. One successful method used is a thermostatically controlled heat exchanger made from fiberglass. If aeration is used, only oil-free air compressors should be used to prevent contamination.

14.6.11.3.2 Before adding seawater to the brine generator, thoroughly clean the generator, aeration supply tube, heater, and any other materials that will be in direct contact with the brine. A good quality biodegradable detergent should be used, followed by several (at least three) thorough deionized water rinses.

14.6.11.3.3 High quality (and preferably high salinity) seawater should be filtered to at least 10 µm before placing into the brine generator. Water should be collected on an incoming tide to minimize the possibility of contamination.

14.6.11.3.4 The temperature of the seawater is increased slowly to 40°C. The water should be aerated to prevent temperature stratification and to increase water evaporation. The brine should be checked daily (depending on the volume being enerated) to ensure that the salinity does not exceed 100‰ and that the temperature does not exceed 40°C. Additional seawater may be added to the brine to obtain the volume of brine required.

14.6.11.3.5 After the required salinity is attained, the HSB should be filtered a second time through a 1 mm filter and poured directly into portable containers (20-L cubitainers or polycarbonate water cooler jugs are suitable). The containers should be capped and labeled with the date the brine was generated and its salinity. Containers of HSB should be stored in the dark and maintained under room temperature until used.

14.6.11.3.6 If a source of HSB is available, test solutions can be made by following the directions below. Thoroughly mix together the deionized water and HSB before mixing in the effluent.

14.6.11.3.7 Divide the salinity of the HSB by the expected test salinity to determine the proportion of deionized water to brine. For example, if the salinity of the brine is 100‰ and the test is to be conducted at 20‰, 100‰ divided by 20‰ = 5.0. The proportion of brine is 1 part in 5 (one part brine to four parts deionized water). To make 1 L of seawater at 20‰ salinity from a HSB of 100‰, 200 mL of brine and 800 mL of deionized water are required.

14.6.11.3.8 Table 2 illustrates the composition of 1800 mL test solutions at 20‰ if they are made by combining effluent (0‰), deionized water and HSB of 100‰ (only). The volume (mL) of brine required is determined by using the amount calculated above. In this case, 200 mL of brine is required for 1 L; therefore, 360 mL would be required for 1.8 L of solution. The volumes of HSB required are constant. The volumes of deionized water are determined by subtracting the volumes of effluent and brine from the total volume of solution: 1800 mL - mL effluent - mL brine = mL deionized water.

14.6.11.4 Artificial sea salts: FORTY FATHOMS® brand sea salts have been used successfully to culture and perform life cycle tests with mysids (Horne, et al., 1983; ASTM, 1993) (see Section 7, Dilution Water). HW MARINEMIX® sea salts have been used successfully to culture mysids and perform the mysid toxicity test (USEPA Region 6 Houston Laboratory; EMSL-Cincinnati). In addition, a slightly modified version of the GP2 medium (Spotte et al., 1984) has been successfully used to perform the mysid survival, growth, and fecundity test (Table 1).

14.6.11.4.1 Synthetic sea salts are packaged in plastic bags and mixed with deionized water or equivalent. The instructions on the package of sea salts should be followed carefully, and the salts should be mixed in a separate container -- not in the culture tank. The deionized water used in hydration should be in the temperature range of 21-26°C. Seawater made from artificial sea salts is conditioned (Spotte, 1973; Spotte, et al., 1984; Bower, 1983) before it is used for culturing or testing. After adding the water, place an airstone in the container, cover, and aerate the solution mildly for 24 h before use.

14.6.11.4.2 The GP2 reagent grade chemicals (Table 1) should be mixed with deionized (DI) water or its equivalent in a container other than the culture or testing tanks. The deionized water used for hydration should be between 21-26°C. The artificial seawater must be conditioned (aerated) for 24 h before use as the testing medium. If the solution is to be autoclaved, sodium bicarbonate is added after the solution has cooled. A stock solution of sodium bicarbonate is made up by dissolving 33.6 g $NaHCO_3$ in 500 mL of deionized water. Add 2.5 mL of this stock solution for each liter of the GP2 artificial seawater.

TABLE 1. REAGENT GRADE CHEMICALS USED IN THE PREPARATION OF GP2 ARTIFICIAL SEAWATER FOR THE MYSID, *MYSIDOPSIS BAHIA*, TOXICITY TEST[1,2,3]

Compound	Concentration (g/L)	Amount (g) Required for 20L
NaCl	21.03	420.6
Na_2SO_4	3.52	70.4
Kcl	0.61	12.2
KBr	0.088	1.76
$Na_2B_4O_7 \cdot 10\ H_2O$	0.034	0.68
$MgCl_2 \cdot 6\ H_2O$	9.50	190.0
$CaCl_2 \cdot 2\ H_2O$	1.32	26.4
$SrCl_2 \cdot 6\ H_2O$	0.02	0.400
$NaHCO_3$	0.17	3.40

1 Modified GP2 from Spotte et al. (1984).
2 The constituent salts and concentrations were taken from USEPA (1990b). The salinity is 30.89 g/L.
3 GP2 can be diluted with deionized (DI) water to the desired test salinity.

14.6.12 BRINE SHRIMP, *ARTEMIA*, NAUPLII -- for feeding cultures and test organisms.

14.6.12.1 Newly hatched *Artemia* nauplii are used for food for the stock cultures and test organisms. Although there are many commercial sources of brine shrimp cysts, the Brazilian or Colombian strains are preferred because the supplies examined have had low concentrations of chemical residues and produce nauplii of suitably small size.

TABLE 2. QUANTITIES OF EFFLUENT, DEIONIZED WATER, AND HYPERSALINE BRINE (100‰) NEEDED TO PREPARE 1800 ML VOLUMES OF TEST SOLUTION WITH A SALINITY OF 20‰

Effluent Concentration (%)	Volume of Effluent (0‰) (mL)	Volume of Deionized Water (mL)	Volume of Hypersaline Brine (mL)	Total Volume (mL)
80	1440	0	360	1800
40	720	720	360	1800
20	360	1080	360	1800
10	180	1260	360	1800
5	90	1350	360	1800
Control	0	1440	360	1800
Total	2790	5850	2160	10800

14.6.12.2 Each new batch of *Artemia* cysts must be evaluated for size (Vanhaecke and Sorgeloos, 1980, and Vanhaecke et al., 1980) and nutritional suitability (Leger, et al., 1985, Leger, et al., 1986) against known suitable reference cysts by performing a side-by-side larval growth test using the "new" and "reference" cysts. The "reference" cysts used in the suitability test may be a previously tested and acceptable batch of cysts. A sample of newly-hatched *Artemia* nauplii from each new batch of cysts should be chemically analyzed. The *Artemia* cysts should not be used if the concentration of total organic chlorine exceeds 0.15 µg/g wet weight or the total concentration of organochlorine pesticides plus PCBs exceeds 0.30 µg/g wet weight (For analytical methods see USEPA, 1982).

14.6.12.2.1 *Artemia* nauplii are obtained as follows:

1 Add 1 L of seawater, or an aqueous uniodized salt (NaCl) solution prepared with 35 g salt or artificial sea salts to 1 L of deionized water, to a 2-L separatory funnel, or equivalent.
2. Add 10 mL *Artemia* cysts to the separatory funnel and aerate for 24 h at 27°C. Hatching time varies with incubation temperature and the geographic strain of *Artemia* used (see USEPA, 1985a; USEPA, 2002a; ASTM, 1993).
3. After 24 h, cut off the air supply in the separatory funnel. *Artemia* nauplii are phototactic, and will concentrate at the bottom of the funnel if it is covered for 5-10 minutes. To prevent mortality, do not leave the concentrated nauplii at the bottom of the funnel more than 10 min without aeration.
4. Drain the nauplii into a beaker or funnel fitted with a 150 µm NITEX® or stainless steel screen, and rinse with seawater or equivalent before use.

14.6.12.3 Testing *Artemia* nauplii as food for toxicity test organisms.

14.6.12.3.1 The primary criteria for acceptability of each new supply of brine shrimp, cysts is adequate survival, growth, and reproduction of the mysids. The mysids used to evaluate the acceptability of the brine shrimp nauplii must be of the same geographical origin and stage of development (7 days old) as those used routinely in the toxicity tests. Sufficient data to detect differences in survival and growth should be obtained by using eight replicate test chambers, each containing 5 mysids, for each type of food.

14.6.12.3.2 The feeding rate and frequency, test vessels, volume of control water, duration of the test, and age of the *Artemia* nauplii at the start of the test, should be the same as used for the routine toxicity tests.

14.6.12.3.3 Results of the brine shrimp, *Artemia*, nauplii nutrition assay, where there are only two treatments, can be evaluated statistically by use of a t test. The "new" food is acceptable if there are no statistically significant differences in the survival, growth, and reproduction of the mysids fed the two sources of nauplii.

14.6.13 TEST ORGANISMS, *Mysidopsis bahia* (see Rodgers et al., 1986 and USEPA, 2002a for information on mysid ecology). The genus name of this organism was formally changed to *Americamysis* (Price *et al.*, 1994); however, the method manual will continue to refer to *Mysidopsis bahia* to maintain consistency with previous versions of the method.

14.6.13.1 Brood Stock

14.6.13.1.1 To provide an adequate supply of juveniles for a test, mysid, *Mysidopsis bahia*, cultures should be started at least four weeks before the test animals are needed. At least 200 mysids, *Mysidopsis bahia*, should be placed in each culture tank to ensure that 1500 to 2000 animals will be available by the time preparations for a test are initiated.

14.6.13.1.2 Mysids, *Mysidopsis bahia*, may be shipped or otherwise transported in polyethylene bottles or CUBITAINERS®. Place 50 animals in 700 mL of seawater in a 1-L shipping container. To control bacterial growth and prevent DO depletion during shipment, do not add food. Before closing the shipping container, oxygenate the water for 10 min. The mysids, *Mysidopsis bahia*, will starve if not fed within 36 h, therefore, they should be shipped so that they are not in transit more than 24 h.

14.6.13.1.3 The identification of the *Mysidopsis bahia* stock culture should be verified using the key from Heard (1982), Price (1978), Price, (1982), Stuck et al. (1979a), and Stuck et al. (1979b). Records of the verification should be retained along with a few of the preserved specimens.

14.6.13.1.4 Glass aquaria (120- to 200-L) are recommended for cultures. Other types of culture chambers may also be convenient. Three or more separate cultures should be maintained to protect against loss of the entire culture stock in case of accident, low DO, or high nitrite levels, and to provide sufficient numbers of juvenile mysids, *Mysidopsis bahia*, for toxicity tests. Fill the aquaria about three-fourths full of seawater. A flow-through system is recommended if sufficient natural seawater is available, but a closed, recirculating or static renewal system may be used if proper water conditioning is provided and care is exercised to keep the pH above 7.8 and nitrite levels below 0.05 mg/L.

14.6.13.1.5 Standard aquarium undergravel filters should be used with either the flow-through or recirculating system to provide aeration and a current conducive to feeding (Gentile et al., 1983). The undergravel filter is covered with a prewashed, coarse (2-5 mm) dolomite substrate, 2.5 cm deep for flow-through cultures or 10 cm deep for recirculating cultures.

14.6.13.1.6 The recirculating culture system is conditioned as follows:

1. After the dolomite has been added, the filter is attached to the air supply and operated for 24 h.
2. Approximately 4 L of seed water obtained from a successfully operating culture is added to the culture chamber.
3. The nitrite level is checked daily with an aquarium test kit or with EPA Method 354.1 (USEPA, 1979b).
4. Add about 30 mL of concentrated *Artemia* nauplii every other day until the nitrite level reaches at least 2.0 mg/L. The nitrite will continue to rise for several days without adding more *Artemia* nauplii and will then slowly decrease to less than 0.05 mg/L.

5. After the nitrite level falls below 0.05 mg/L, add another 30 mL of *Artemia* nauplii concentrate and check the nitrite concentration every day.
6. Continue this cycle until the addition of *Artemia* nauplii does not cause a rise in the nitrite concentration. The culture chamber is then conditioned and is ready to receive mysids.
7. Add only a few (5-20) mysids at first, to determine if conditions are favorable. If these mysids are still doing well after a week, several hundred more can be added.

14.6.13.1.7 It is important to add enough food to keep the adult animals from cannibalizing the young, but not so much that the DO is depleted or that there is a buildup of toxic concentrations of ammonia and nitrite. Just enough newly-hatched *Artemia* nauplii are fed twice a day so that each feeding is consumed before the next feeding.

14.6.13.1.8 Natural seawater is recommended as the culture medium, but HSB may be used to make up the culture water if natural seawater is not available. EMSL-Cincinnati has successfully used FORTY FATHOMS® artificial sea salts for culturing and toxicity tests of mysids, and USEPA, Region 6 has used HW MARINEMIX® artificial sea salts.

14.6.13.1.9 Mysids, *Mysidopsis bahia*, should be cultured at a temperature of 26 ± 1°C. No water temperature control equipment is needed if the ambient laboratory temperature remains in the recommended range, and if there are no frequent, rapid, large temperature excursions in the culture room.

14.6.13.1.10 The salinity should be maintained at 30 ± 2‰, or at a lower salinity (but not less than 20‰) if most of the tests will be conducted at a lower salinity.

14.6.13.1.11 Day/night cycles prevailing in most laboratories will provide adequate illumination for normal growth and reproduction. A 16-h/8-h day/night cycle in which the light is gradually increased and decreased to simulate dawn and dusk conditions, is recommended.

14.6.13.1.12 Mysid, *Mysidopsis bahia*, culture may suffer if DOs fall below 5 mg/L for extended periods. The undergravel filter will usually provide sufficient DO. If the DO drops below 5 mg/L at 25°C and 30‰, additional aeration should be provided. Measure the DO in the cultures daily the first week and then at least weekly thereafter.

14.6.13.1.13 Suspend a clear glass or plastic panel over the cultures, or use some other means of excluding dust and dirt, but leave enough space between the covers and culture tanks to allow circulation of air over the cultures.

14.6.13.1.14 If hydroids or worms appear in the cultures, remove the mysids and clean the chambers thoroughly, using soap and hot water. Rinse once with acid (10% HCl) and three times with distilled or deionized water. Mysids with attached hydroids should be discarded. Those without hydroids should be transferred by hand pipetting into three changes of clean seawater before returning them to the cleaned culture chamber. To guard against predators, natural seawater should be filtered through a net with 30 µm mesh openings before entering the culture vessels.

14.6.13.1.15 Mysids, *Mysidopsis bahia*, are very sensitive to low pH and sudden changes in temperature. Care should be taken to maintain the pH at 8.0 ± 0.3, and to limit rapid changes in water temperature to less than 3°C.

14.6.13.1.16 Mysids, *Mysidopsis bahia*, should be handled carefully and as little as possible so that they are not unnecessarily stressed or injured. They should be transferred between culture chambers with long handled cups with netted bottoms. Animals should be transferred to the test vessels with a large bore pipette (4-mm), taking care to release the animals under the surface of the water. Discard any mysids that are injured during handling.

14.6.13.1.17 Culture Maintenance (Also See USEPA, 2002a)

14.6.13.1.17.1 Cultures in closed, recirculating systems are fed twice a day. If no nauplii are present in the culture chamber after four hours, the amount of food should be increased slightly. In flow-through systems, excess food can be a problem by promoting bacterial growth and low dissolved oxygen.

14.6.13.1.17.2 Careful culture maintenance is essential. The organisms should not be allowed to become too crowded. The cultures should be cropped as often as necessary to maintain a density of about 20 mysids per liter. At this density, at least 70% of the females should have eggs in their brood pouch. If they do not, the cultures are probably under stress, and the cause should be found and corrected. If the cause cannot be found, it may be necessary to restart the cultures with a clean culture chamber, a new batch of culture water, and clean gravel.

14.6.13.1.17.3 In closed, recirculating systems, about one third of the culture water should be replaced with newly prepared seawater every week. Before siphoning the old media from the culture, it is recommended that the sides of the vessel be scraped and the gravel carefully turned over to prevent excessive buildup of algal growth. Twice a year the mysids should be removed from the recirculating cultures, the gravel rinsed in clean seawater, the sides of the chamber washed with clean seawater, and the gravel and animals returned to the culture vessel with newly conditioned seawater. No detergent should be used, and care should be taken not to rinse all the bacteria from the gravel.

14.6.13.2 Test Organisms

14.6.13.2.1 The test is begun with 7-day-old juveniles. To have the test animals available and acclimated to test conditions at the start of the test, gravid females must be obtained from the stock culture eight days in advance of the test. Whenever possible, brood stock should be obtained from cultures having similar salinity, temperature, light regime, etc., as are to be used in the toxicity test.

14.6.13.2.2 Eight days before the test is to start, sufficient gravid females are placed in brood chambers. Assuming that 240 juveniles are needed for each test, approximately half this number (120) of gravid females should be transferred to brood chambers. The mysids are removed from the culture tank with a net or netted cup and placed in 20-cm diameter finger bowls. The gravid females are transferred from the finger bowls to the brood chambers with a large-bore pipette or, alternatively, are transferred by pouring the contents of the finger bowls into the water in the brood chambers.

14.6.13.2.3 The mysid juveniles may be collected for the toxicity tests by transferring gravid females from the stock cultures to netted (1000 µm) flow-through containers (Figure 1) held within 4-L glass, wide-mouth separatory funnels. Newly released juveniles can pass through the netting, whereas the females are retained. The gravid females are fed newly hatched *Artemia* nauplii, and are held overnight to permit the release of young. The juvenile mysids are collected by opening the stopcock on the funnel and collecting them in another container from which they are transferred to holding tanks using a wide bore (4 mm ID) pipette. The brood chambers usually require aeration to maintain sufficient DO and to keep the food in suspension.

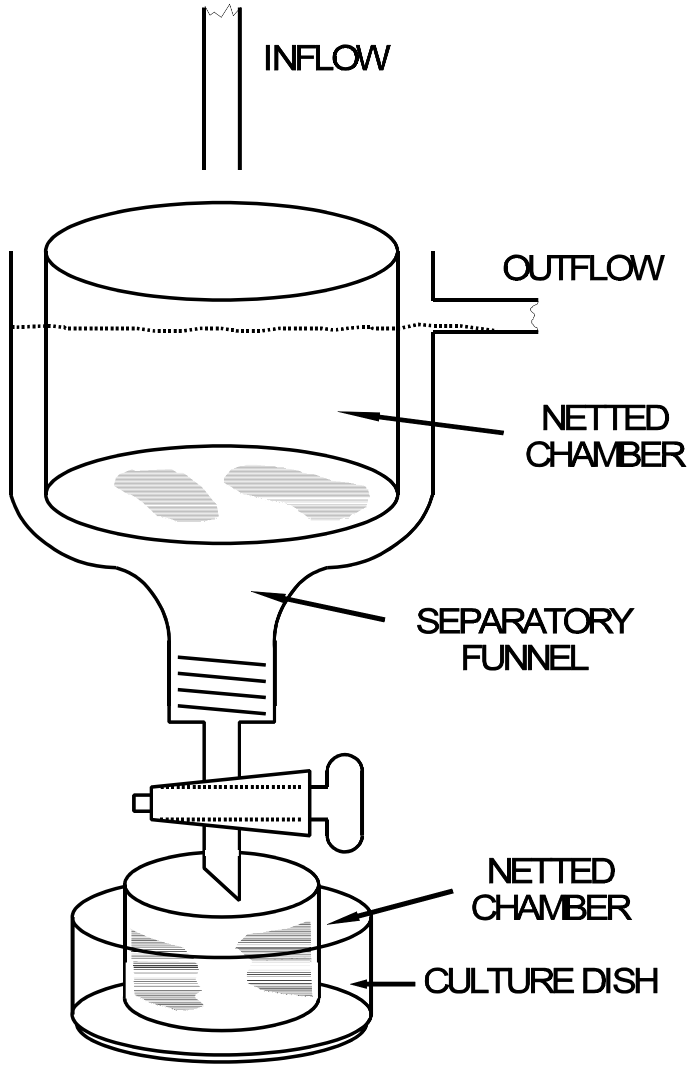

Figure 1. Apparatus (brood chamber) for collection of juvenile mysids, *Mysidopsis bahia*. From USEPA (1987d).

14.6.13.2.4 The temperature in the brood chamber should be maintained at the upper acceptable culture limit (26 - 27°C), or 1°C higher than the cultures, to encourage faster brood release. At this temperature, sufficient juveniles should be produced for the test.

14.6.13.2.5 The newly released juveniles (age = 0 days) are transferred to 20-L glass aquaria (holding vessels) which are gently aerated. Smaller holding vessels may be used, but the density of organisms should not exceed 10 mysids per liter. The test animals are held in the holding vessel for six days prior to initiation of the test. The holding medium is renewed every other day.

14.6.13.2.6 During the holding period, the mysids are acclimated to the salinity at which the test will be conducted, unless already at that salinity. The salinity should be changed no more than 2‰ per 24 h to minimize stress on the juveniles.

14.6.13.2.7 The temperature during the holding period is critical to mysid development, and must be maintained at 26 ± 1°C. If the temperature cannot be maintained in this range, it is advisable to hold the juveniles an additional day before beginning the test.

14.6.13.2.8 During the holding period, just enough newly-hatched *Artemia* nauplii are fed twice a day (a total of at least 150 nauplii per mysid per day) so that some food is constantly present.

14.6.13.2.9 If the test is to be performed in the field, the juvenile mysids, *Mysidopsis bahia*, should be gently siphoned into 1-L polyethylene wide-mouth jars with screw-cap lids filled two-thirds full with clean seawater from the holding tank. The water in these jars is aerated for 10 min, and the jars are capped and packed in insulated boxes for shipment to the test site. Food should not be added to the jars to prevent the development of excessive bacterial growth and a reduction in DO.

14.6.13.2.10 Upon arrival at the test site (in less than 24 h) the mysids, *Mysidopsis bahia*, are gently poured from the jars into 20-cm diameter glass culture dishes. The jars are rinsed with salt water to dislodge any mysids that may adhere to the sides. If the water appears milky, siphon off half of it with a netted funnel (to avoid siphoning the mysids) and replace with clean salt water of the same salinity and temperature. If no *Artemia* nauplii are present in the dishes, feed about 150 *Artemia* nauplii per mysid.

14.6.13.2.11 The pre-test holding conditions of test organisms (as well as the test conditions) have been shown to significantly influence the success of achieving the test acceptability criteria for the fecundity endpoint (egg production by 50% or more of control females). Temperature, feeding, and organism density are important factors in the rate of mysid development. Laboratories should optimize these factors (within the limits of the test procedure) during both the pre-test holding period and the testing period to encourage achieving the test acceptability criteria for the fecundity endpoint. If test organisms are purchased, the testing laboratory should also confer with the supplier to ensure that pre-test holding conditions are optimized to successfully achieve the fecundity endpoint. Lussier *et al.* (1999) found that by increasing holding temperature and test temperature from 26°C ± 1°C to 26°C - 27°C and maintaining holding densities to ≤10 organisms / L, the percentage of tests meeting the test acceptability criteria for fecundity increased from 60% to 97%. While the fecundity endpoint is an optional endpoint, it is often the most sensitive measure of toxicity, and the 7-d mysid test estimates the chronic toxicity of effluents most effectively when all three endpoints (survival, growth, and fecundity) are measured (Lussier *et al.* 1999).

14.7 EFFLUENT AND RECEIVING WATER COLLECTION, PRESERVATION, AND STORAGE

14.7.1 See Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests.

14.8 CALIBRATION AND STANDARDIZATION

14.8.1 See Section 4, Quality Assurance.

14.9 QUALITY CONTROL

14.9.1 See Section 4, Quality Assurance.

14.9.2 The reference toxicant recommended for use with the mysid 7-day test is copper sulfate or sodium dodecyl sulfate.

14.10 TEST PROCEDURES

14.10.1 TEST DESIGN

14.10.1.1 The test consists of at least five effluent concentrations plus a site water control and a reference water treatment (natural seawater or seawater made up from hypersaline brine, or equivalent).

14.10.1.2 Effluent concentrations are expressed as percent effluent.

14.10.1.3 Eight replicate test vessels, each containing 5 to 7 day old animals, are used per effluent concentration and control.

14.10.2 TEST SOLUTIONS

14.10.2.1 Receiving waters

14.10.2.1.1 The sampling point(s) is determined by the objectives of the test. At estuarine and marine sites, samples are usually collected at mid-depth. Receiving water toxicity is determined with samples used directly as collected or with samples passed through a 60 µm NITEX® filter and compared without dilution, against a control. Using eight replicate chambers per test, each containing 150 mL, and 400 mL for chemical analysis, would require approximately 1.6 L or more of sample per test per day.

14.10.2.2 Effluents

14.10.2.2.1 The selection of the effluent test concentrations should be based on the objectives of the study. A dilution factor of 0.5 is commonly used. A dilution factor of 0.5 provides precision of ± 100%, and testing of concentrations between 6.25% and 100% effluent using only five effluent concentrations (6.25%, 12.5%, 25%, 50%, and 100%). Test precision shows little improvement as dilution factors are increased beyond 0.5 and declines rapidly if smaller dilution factors are used. **Therefore, USEPA recommends the use of the ≥ 0.5 dilution factor.** If 100‰ HSB is used as a diluent, the maximum concentration of effluent that can be tested will be 80% at 20‰ and 70% at 30‰ salinity.

14.10.2.2.2 If the effluent is known or suspected to be highly toxic, a lower range of effluent concentrations should be used (such as 25%, 12.5%, 6.25%, 3.12%, and 1.56%). If high mortality is observed during the first 1-to-2 h of the test, additional dilutions at the lower range of effluent concentrations should be added.

14.10.2.2.3 The volume of effluent required for daily renewal of eight replicates per concentration for five concentrations of effluent and a control, each containing 150 mL of test solution, is approximately 1200 mL. Prepare enough test solution (approximately 1600 mL) at each effluent concentration to provide 400 mL additional volume for chemical analyses.

14.10.2.2.4 Just prior to test initiation (approximately 1 h), the temperature of a sufficient quantity of the sample to make the test solutions should be adjusted to the test temperature (26 ± 1°C) and maintained at that temperature during the addition of dilution water.

14.10.2.2.5 Higher effluent concentrations (i.e., 25%, 50%, and 100%) may require aeration to maintain adequate dissolved oxygen concentrations. However, if one solution is aerated, all concentrations must be aerated. Aerate effluent as it warms and continue to gently aerate test solutions in the test chambers for the duration of the test.

14.10.2.2.6 Effluent dilutions should be prepared for all replicates in each treatment in one flask to minimize variability among the replicates. The test chambers (cups) are labeled with the test concentration and replicate number. Dispense 150 mL of the appropriate effluent dilution to each test chamber.

14.10.2.3 Dilution Water

14.10.2.3.1 Dilution water may be uncontaminated natural seawater (receiving water), HSB prepared from natural seawater, or artificial seawater prepared from FORTY FATHOMS® or GP2 sea salts (see Table 1 and Section 7, Dilution Water). Other artificial sea salts may be used for culturing mysid and for the survival, growth, and fecundity test if the control criteria for acceptability of test data are satisfied.

14.10.3 START OF THE TEST

14.10.3.1 The test should begin as soon as possible, preferably within 24 h after sample collection. The maximum holding time following retrieval of the sample from the sampling device should not exceed 36 h for off-site toxicity tests unless permission is granted by the permitting authority. In no case should the test be started more than 72 h after sample collection (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests).

14.10.3.2 Begin the test by randomly placing five animals (one at a time) in each test cup of each treatment using a large bore (4 mm ID) pipette (see Appendix A for an example of randomization). It is easier to capture the animals if the volume of water in the dish is reduced and the dish is placed on a light table. It is recommended that the transfer pipette be rinsed frequently because mysids tend to adhere to the inside surface.

14.10.4 LIGHT, PHOTOPERIOD, SALINITY AND TEMPERATURE

14.10.4.1 The light quality and intensity under ambient laboratory conditions are generally adequate. Light intensity of 10-20 $\mu E/m^2/s$, or 50 to 100 foot candles (ft-c), with a photoperiod of 16 h light and 8 h darkness. It is critical that the test water temperature be maintained at $26 \pm 1°C$. It is recommended that the test water temperature be continuously recorded. The salinity should vary no more than ± 2‰ among chambers on a given day. If effluent and receiving water tests are conducted concurrently, the salinities of these tests should be similar.

14.10.4.1.1 If a water bath is used to maintain the test temperature, the water depth surrounding the test cups should be at least 2.5 cm deep.

14.10.4.1.2 Rooms or incubators with high volume ventilation should be used with caution because the volatilization of the test solutions and evaporation of dilution water may cause wide fluctuations in salinity. Covering the test cups with clear polyethylene plastic may help prevent volatilization and evaporation of the test solutions.

14.10.5 DISSOLVED OXYGEN (DO) CONCENTRATION

14.10.5.1 Aeration may affect the toxicity of effluents and should be used only as a last resort to maintain a satisfactory DO. The DO should be measured on new solutions at the start of the test (Day 0) and before daily renewal of test solutions on subsequent days. The DO should not fall below 4.0 mg/L (see Section 8, Effluent and Receiving Water Sampling, Sample Handling, and Sample Preparation for Toxicity Tests). If it is necessary to aerate, all treatments and the control should be aerated. The aeration rate should not exceed 100 bubbles/minute, using a pipet with a 1-2 mm orifice, such as a 1-mL KIMAX® serological pipet, or equivalent. Care should be taken to ensure that turbulence resulting from aeration does not cause undue stress on the mysid.

14.10.6 FEEDING

14.10.6.1 *Artemia* nauplii are prepared as described above.

14.10.6.2 During the test, the mysids in each test chamber should be fed *Artemia* nauplii, (less than 24-h old), at the rate of 150 nauplii per mysid per day. Adding the entire daily ration at a single feeding immediately after test solution renewal may result in a significant DO depression. Therefore, it is preferable to feed half of the daily ration immediately after test solution renewal, and the second half 8 - 12 h later. Increase the feeding if the nauplii are consumed in less than 4 h. It is important that the nauplii be washed before introduction to the test chamber.

14.10.7 DAILY CLEANING OF TEST CHAMBERS

14.10.7.1 Before the daily renewal of test solutions, uneaten and dead *Artemia*, dead mysids and other debris are removed from the bottom of the test chambers with a pipette. As much of the uneaten *Artemia* as possible should be removed from each chamber to ensure that the mysids principally eat new hatched nauplii. By placing the test chambers on a light box, inadvertent removal of live mysids can be greatly reduced because they can be more easily seen. Any incidence of removal of live mysids from the test chambers during cleaning, and subsequent return to the chambers should be noted in the test records.

14.10.8 OBSERVATIONS DURING THE TEST

14.10.8.1 Routine Chemical and Physical Determinations

14.10.8.1.1 DO is measured at the beginning and end of each 24-h exposure period in one test chamber at each test concentration and in the control.

14.10.8.1.2 Temperature, pH, and salinity are measured at the end of each 24-h exposure period in one test chamber at each concentration and in the control. Temperature should also be monitored continuously observed and recorded daily for at least two locations in the environmental control system or the samples. Temperature should be measured in a sufficient number of test chambers at least at the end of the test to determine temperature variation in environmental chamber.

14.10.8.1.3 The pH is measured in the effluent sample each day before new test solutions are made.

14.10.8.2 Routine Biological Observations

14.10.8.2.1 The number of live mysids are counted and recorded each day when the test solutions are renewed (Figure 7). Dead animals and excess food should be removed with a pipette before test solutions are renewed.

14.10.8.2.2 Protect the mysids from unnecessary disturbance during the test by carrying out the daily test observations, solution renewals, and removal of the dead mysids, carefully. Make sure the mysids remain immersed during the performance of the above operations.

14.10.9 TEST SOLUTION RENEWAL

14.10.9.1 Before the daily renewal of test solutions, slowly pour off all but 10 mL of the old test medium into a 20 cm diameter culture dish on a light table. Be sure to check for animals that may have adhered to the sides of the test chamber. Rinse them back into the test cups. Add 150 mL of new test solution slowly to each cup. Check the culture dish for animals that may have been poured out with the old media, and return them to the test chamber.

14.10.10 TERMINATION OF THE TEST

14.10.10.1 After measuring the DO, pH, temperature, and salinity and recording survival, terminate the test by pouring off the test solution in all the cups to a one cm depth and refilling the cups with clean seawater. This will keep the animals alive, but not exposed to the toxicant, while waiting to be examined for sex and the presence of eggs.

14.10.10.2 The live animals must be examined for eggs and the sexes determined within 12 h of the termination of the test. If the test was conducted in the field, and the animals cannot be examined on site, the live animals should be shipped back to the laboratory for processing. Pour each replicate into a labeled 100 mL plastic screw capped jar, and send to the laboratory immediately.

14.10.10.3 If the test was conducted in the laboratory, or when the test animals arrive in the laboratory from the field test site, the test organisms must be processed immediately while still alive as follows:

14.10.10.3.1 Examine each replicate under a stereomicroscope (240X) to determine the number of immature animals, the sex of the mature animals, and the presence or absence of eggs in the oviducts or brood sacs of the females (see Figures 3-6). This must be done while the mysids are alive because they turn opaque upon dying. This step should not be attempted by a person who has not had specialized training in the determination of sex and presence of eggs in the oviduct. NOTE: Adult females without eggs in the oviduct or brood sac look like immature mysids (see Figure 6).

TEST: ____________________

START DATE: ____________________

SALINITY: ____________________

	TRTMT	TEMP	SALINITY	D.O.	pH	TRTMT	TEMP	SALINITY	D.O.	pH	
DAY 1	REP										
	REP										
DAY 2	REP										
	REP										
DAY 3	REP										
	REP										
DAY 4	REP										
	REP										
DAY 5	REP										
	REP										
DAY 6	REP										
	REP										
DAY 7	REP										
	REP										

	TRTMT	TEMP	SALINITY	D.O.	pH	TRTMT	TEMP	SALINITY	D.O	pH	
DAY 1	REP										
	REP										
DAY 2	REP										
	REP										
DAY 3	REP										
	REP										
DAY 4	REP										
	REP										
DAY 5	REP										
	REP										
DAY 6	REP										
	REP										
DAY 7	REP										
	REP										

Figure 2. Data form for the mysid, *Mysidopsis bahia*, water quality measurements. From USEPA (1987d).

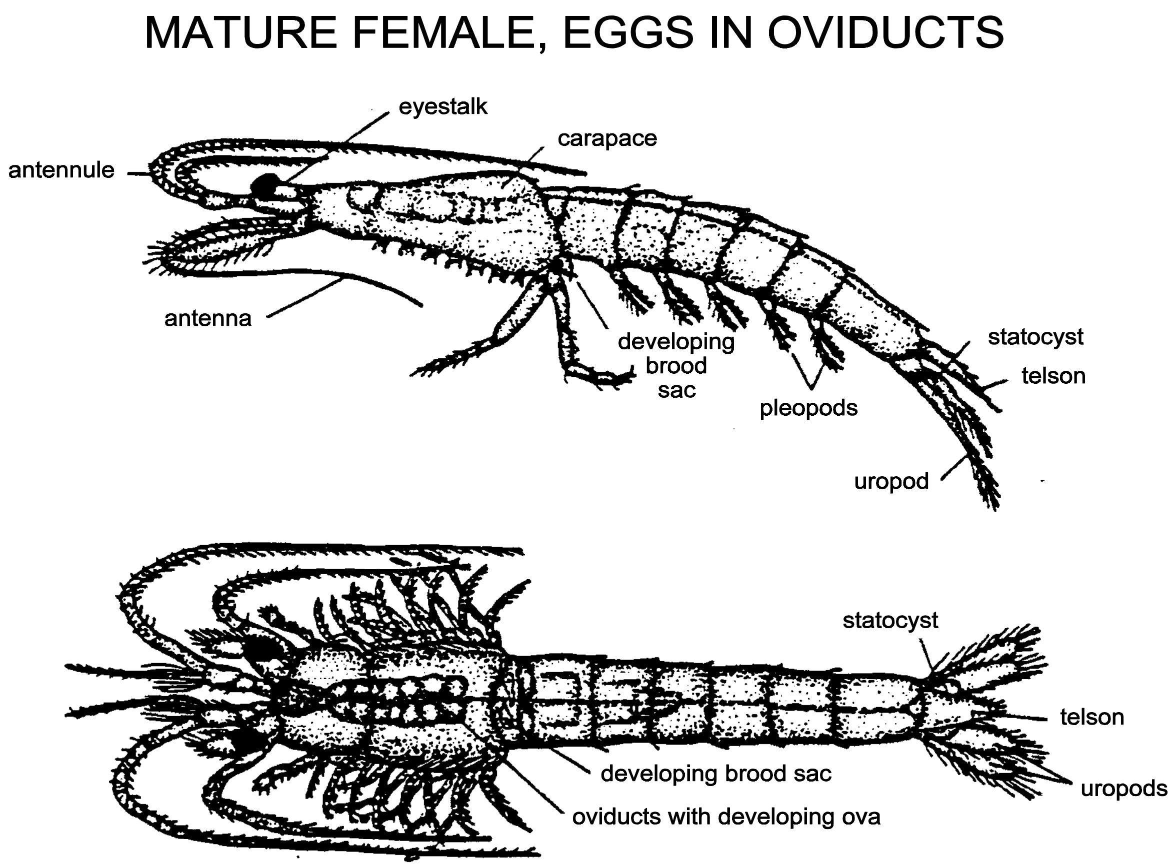

Figure 3. Mature female mysid, *Mysidopsis bahia*, with eggs in oviducts. From USEPA (1987d).

MATURE FEMALE, EGGS IN BROOD SAC

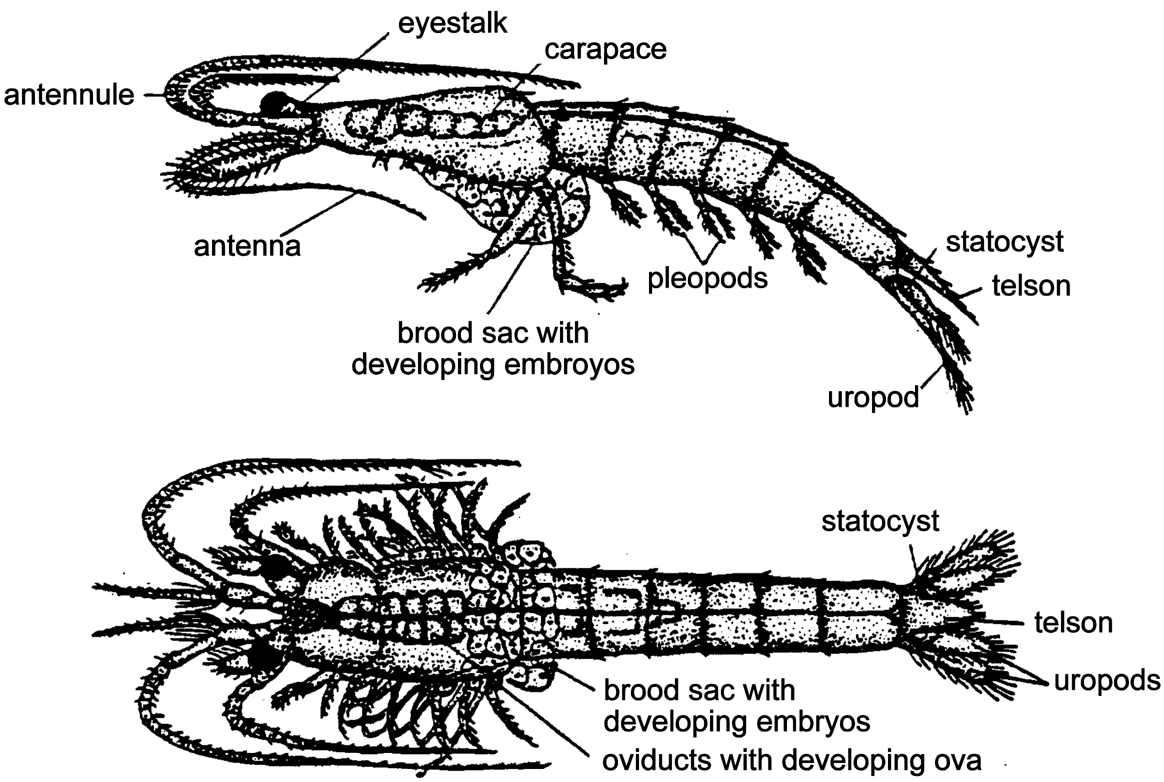

Figure 4. Mature female mysid, *Mysidopsis bahia*, with eggs in oviducts and developing embryos in the brood sac. Above: lateral view. Below: dorsal view. From USEPA (1987d).

14.10.10.3.2   Record the number of immatures, males, females with eggs and females without eggs on data sheets (Figure 7).

14.10.10.3.3   Rinse the mysids by pipetting them into a small netted cup and dipping the cup into a dish containing deionized water. Using forceps, place the mysids from each replicate cup on tared weighing boats and dry at 60°C for 24 h or at 105°C for at least 6 h.

14.10.10.3.4   Immediately upon removal from the drying oven, the weighing pans were placed in a dessicator until weighed, to prevent absorption of moisture from the air. Weigh to the nearest mg. Record weighing pans and subtract the tare weight to determine the dry weight of the mysid in each replicate. Record the weights (Figure 8). For each test chamber, divide the first dry weight by the number of original mysids per replicate to determine the average individual dry weight and record data. For the controls also calculate the mean weight per surviving mysid in the test chamber to evaluate if weights met test acceptability criteria (see Subsection 14.12).

14.10.9.3.5   Pieces of aluminum foil (1-cm square) or small aluminum weighing pans can be used for dry weight analyses. The weighing pans should not exceed 10 mg in weight.

14.10.9.3.6   Number each pan with a waterproof pen with the treatment concentration and replicate number. Forty-eight (48) weigh pans are required per test if all the organisms survive.

MATURE MALE

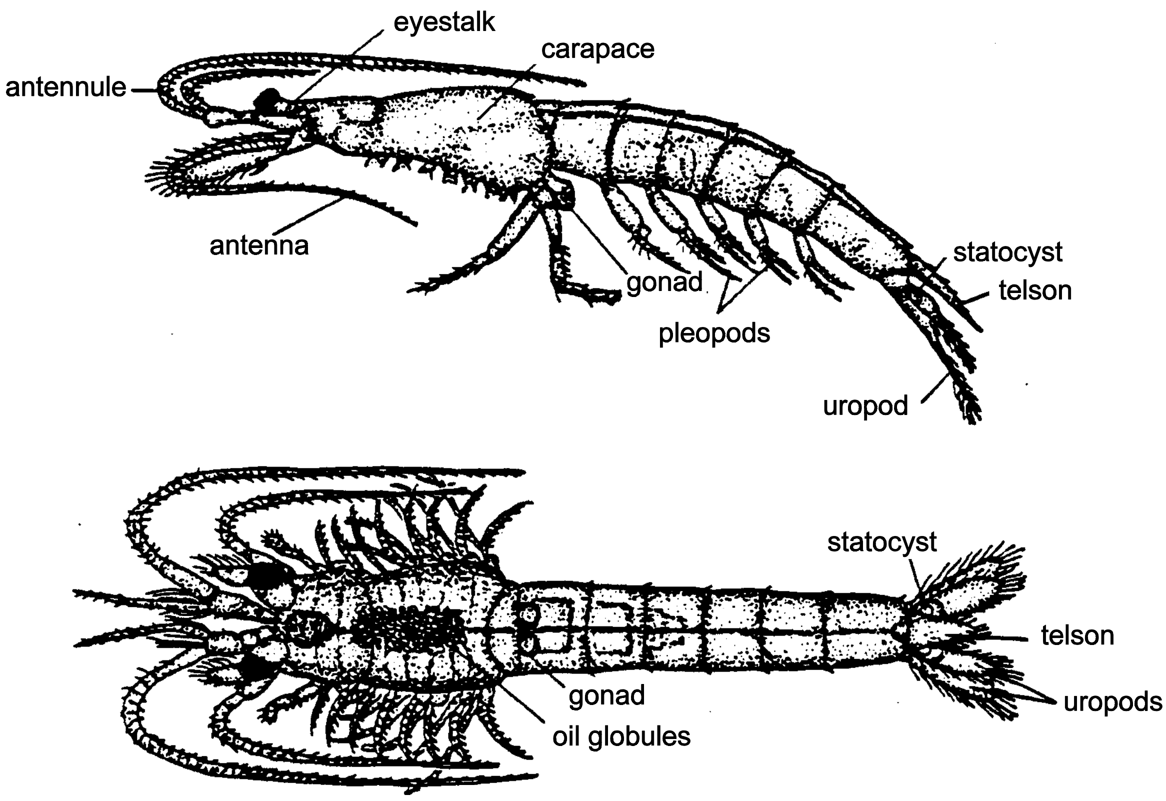

Figure 5. Mature male mysid, *Mysidopsis bahia*. From USEPA (1987d).

14.11 SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA

14.11.1 A summary of test conditions and test acceptability criteria is listed in Table 3.

14.12 ACCEPTABILITY OF TEST RESULTS

14.12.1 The minimum requirements for an acceptable test are 80% survival and an average weight of at least 0.20 mg/surviving mysid in the controls. If fecundity in the controls is adequate (egg production by 50% of females), fecundity should be used as a criterion of effect in addition to survival and growth.

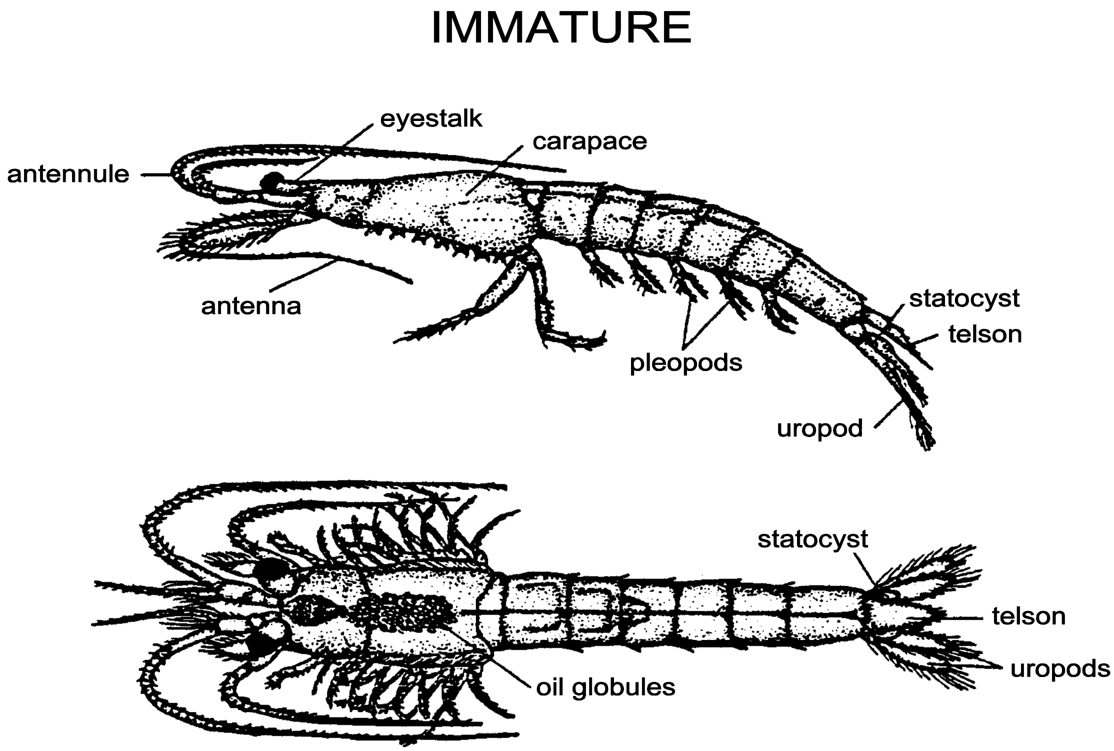

Figure 6. Immature mysid, *Mysidopsis bahia*, (A) lateral view, (B) dorsal view. From USEPA (1987d).

14.13 DATA ANALYSIS

14.13.1 GENERAL

14.13.1.1 Tabulate and summarize the data. Table 4 presents a sample set of survival, growth, and fecundity data.

14.13.1.2 The endpoints of the mysid 7-day chronic test are based on the adverse effects on survival, growth, and egg development. The LC50, the IC25, and the IC50 are calculated using point estimation techniques (see Section 9, Chronic Toxicity Test Endpoints and Data Analysis). LOEC and NOEC values for survival, growth, and fecundity are obtained using a hypothesis testing approach such as Dunnett's Procedure (Dunnett, 1955) or Steel's Many-one Rank Test (Steel, 1959; Miller, 1981) (see Section 9). Separate analyses are performed for the estimation of the LOEC and NOEC endpoints and for the estimation of the LC50, IC25, and IC50. Concentrations at which there is no survival in any of the test chambers are excluded from the statistical analysis of the NOEC and LOEC for survival, growth, and fecundity, but included in the estimation of the LC50, IC25, and IC50. See the Appendices for examples of the manual computations, and examples of data input and program output.

14.13.1.3 The statistical tests described here must be used with a knowledge of the assumptions upon which the tests are contingent. The assistance of a statistician is recommended for analysts who are not proficient in statistics.

TEST: ______________________________

START DATE: ________________________

SALINITY: __________________________

TREATMENT/ REPLICATE	DAY 1 # ALIVE	DAY 2 # ALIVE	DAY 3 # ALIVE	DAY 4 # ALIVE	DAY 5 # ALIVE	DAY 6 # ALIVE	DAY 7 # ALIVE	FEMALES W/EGGS	FEMALES NO EGGS	MALES	IMMATURES
C											
1											
2											

Figure 7. Data form for the mysid, *Mysidopsis bahia*, survival and fecundity data. From USEPA (1987d).

TEST: ______________________________

START DATE: ________________________

SALINITY: ___________________________

TREATMENT/ REPLICATE		DAY 1 # ALIVE	DAY 2 # ALIVE	DAY 3 # ALIVE	DAY 4 # ALIVE	DAY 5 # ALIVE	DAY 6 # ALIVE	DAY 7 # ALIVE	FEMALES W/EGGS	FEMALES NO EGGS	MALES	IMMATURES
	1											
	2											
	3											
3	4											
	5											
	6											
	7											
	8											
	1											
	2											
	3											
4	4											
	5											
	6											
	7											
	8											
	1											
	2											
	3											
5	4											
	5											
	6											
	7											
	8											

Figure 7. Data form for the mysid, *Mysidopsis bahia*, survival and fecundity data (CONTINUED). From USEPA (1987d).

TEST: ______________________________

START DATE: ________________________

SALINITY: ___________________________

TREATMENT/REPLICATE		PAN #	TARE WT.	TOTAL WT.	ANIMAL WT.	# OF ANIMALS	WT./ANIMAL
	1						
	2						
	3						
C	4						
	5						
	6						
	7						
	8						
	1						
	2						
	3						
1	4						
	5						
	6						
	7						
	8						
	1						
	2						
	3						
2	4						
	5						
	6						
	7						
	8						

Figure 8. Data form for the mysid, *Mysidopsis bahia*, dry weight measurements. From USEPA (1987d).

TEST: ______________________________

START DATE: ______________________

SALINITY: _________________________

TREATMENT/REPLICATE		PAN #	TARE WT.	TOTAL WT.	ANIMAL WT.	# OF ANIMALS	WT./ANIMAL
	1						
	2						
	3						
3	4						
	5						
	6						
	7						
	8						
	1						
	2						
	3						
4	4						
	5						
	6						
	7						
	8						
	1						
	2						
	3						
5	4						
	5						
	6						
	7						
	8						

Figure 8. Data form for the mysid, *Mysidopsis bahia*, dry weight measurements (CONTINUED). From USEPA (1987d).